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Amplified detection of femtomolar DNA based on a one-to-few recognition reaction between DNA–Au conjugate and target DNA†

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A sensitive electrochemical DNA biosensor based on the amplification of Au nanoparticles (AuNPs) has been developed. The AuNPs were modified with two types of signaling reporter DNAs, *i.e.* a methylene blue probe (MB-probe 2-SH) and T10 with a methylene blue signaling molecule (MB-T10-SH), forming DNA–AuNP conjugates. The MB-probe 2-SH is complementary to the target DNA, while MB-T10-SH is not. The presence of MB-T10-SH reduces the cross-reaction between target DNA and MB-probe 2-SH on the AuNPs, resulting in increased sensitivity of the biosensor. In our assay, the DNA sensor is fabricated by immobilizing a capture probe on the surface of the Au electrode, which then hybridizes with the corresponding target DNA, and further hybridizes with a DNA–Au conjugate. The signal of MB is measured by differential pulse voltammetry, while the DNA–Au conjugate enables the detection of target DNA in the linear range of 10^{-13} to 10^{-8} M with the detection limit as low as 50 fM.

Introduction

Sensitive and selective detection of mutations and damaged DNA bases is emerging as one of the most important research areas due to its wide applications in clinical medicine,[1] experimental biology[2] and environmental monitoring.[3] Until now, a variety of methods, such as optical,[4,5] fluorescence[6] and electrochemical ones,[2,7–11] have been applied in DNA sensors. Among them, the electrochemical detection has attracted a lot of interest since it is portable and inexpensive.[2,7–10] Usually, an electrochemical DNA sensor consists of a solid electrode and a surface-confined capture probe. After hybridization with the sequence-specific target DNA, the reporting molecules, such as intercalators,[11,12] intercalate with the hybridized double-stranded DNA or are covalently tagged to DNA strands that can generate the electrochemical signals.[13]

In order to improve the sensitivity of DNA sensors, various strategies have been proposed. For example, DNAzyme has been reported to detect DNA and a significant improvement of the detection limit has been achieved.[14,15] In addition, inorganic nanoparticles[13,16,17] and carbon materials[16,18] have been chosen as redox labels to detect DNA due to their high stability, large surface-to-volume ratio, low cost and labeling convenience.[19] In particular, the DNA–Au nanoparticle (AuNP) bio-barcode has been widely used due to its effective amplification based on the functionalized AuNP with a large number of oligonucleotide strands.[13,19–21]

In this contribution, an AuNP is functionalized with a signaling reporter, *i.e.* a methylene blue probe (MB-probe 2-SH), *via* the Au–thiol bonding to form the MB-probe 2-AuNP conjugate. Since many MB-probe 2-SH molecules can accommodate on the surface of one AuNP, one MB-probe 2-AuNP conjugate can hybridize with many target DNA molecules. Normally, for the same number of target DNA molecules assembled on the electrode surface, this one-to-many interaction between the MB-probe 2-AuNP conjugate and target DNA will reduce the quantity of MB-probe 2-AuNP conjugates compared to the case of one MB-probe 2-AuNP hybridizing with only one or few target DNA.[8] Consequently, the electrochemical signal will decrease, resulting in the decrease of detection sensitivity.

One method to solve the aforementioned problem is to introduce spacers between MB-probe 2-SH molecules in the MB-probe 2-AuNP conjugate, which can dilute the attachment of the MB-probe 2-SH on the AuNP surface and hence increase the

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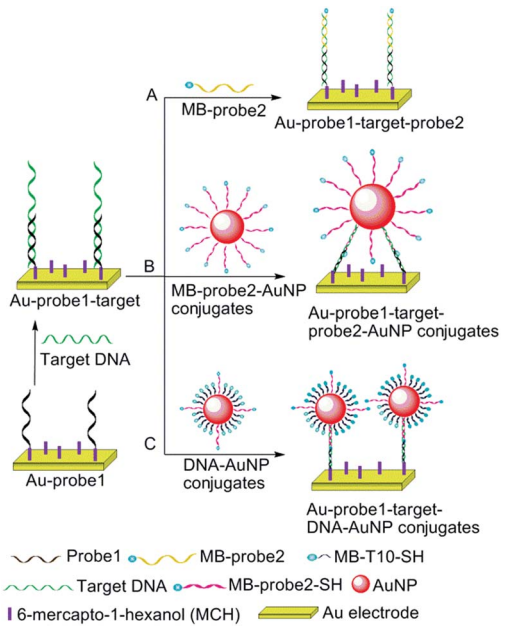
quantity of MB-probe 2-AuNP conjugates for the same amount of target DNA. Unfortunately, the signal might decrease due to the decrease of MB-probe 2-SH on the AuNP surface. In order to maintain or even improve the electrochemical signal, a kind of spacer with the same signaling molecule, *i.e.* T10 with an MB signaling molecule (MB-T10-SH), will be introduced to form the DNA–AuNP conjugate. The presence of MB-T10-SH will replace some of the MB-probe 2-SH molecules on the surface of AuNP, which helps reduce the cross-reaction between MB-probe 2-AuNP conjugates and target DNA. It means that the recognition reaction between the DNA–AuNP conjugate and target DNA decreased from one-to-many to one-to-few. Therefore, the quantity of hybridization events between DNA–AuNP conjugates and target DNA will increase for the same number of target DNA on the electrode surface compared to the number of hybridization events between MB-probe 2-AuNP conjugates and target DNA. At the same time, the electrochemical signal will be improved since the density of MB-T10-SH is increased due to the rodlike conjugation of MB-T10-SH on the AuNP surface compared to the MB-probe 2-SH.[22] Therefore, the MB-T10-SH plays roles of a signaling reporter and spacer.

A "sandwich-type" detection strategy was employed in our design (Scheme 1). All the sequences of oligonucleotides shown in Scheme 1 are listed in Table 1. First, the capture probe was conjugated onto the Au electrode surface. Once the conjugation was stabilized and the sample was rinsed, drops of target DNA solution with different concentrations were added onto the modified electrode and incubated for 1 h at room temperature. The putative target-captured electrode surface was subsequently hybridized with any one of the following probe conjugates, MB-probe 2, MB-probe 2-AuNP conjugates or DNA–AuNP conjugates. The results showed that the last conjugate provided the greatest sensitivity of femtomolar target DNA and excellent differentiation ability for single-nucleotide polymorphisms (SNPs). Here, methylene blue (MB) was employed as the signaling molecule. Since the signal of MB is proportional to the amount of reporter DNA and further corresponds to the concentration of target DNA, the detection of target DNA is thus realized. Compared to the previous reports where the signaling molecule of MB was inserted to the system based on the electrostatic interaction between MB and DNA strains,[8,23] the covalent bonding between MB and DNA in the present report is more stable. Consequently, the signal will be maintained for a long time. In addition, the signal will be significantly amplified with DNA-modified AuNPs (~13 nm), which have an average 100 bar codes loaded onto the surface.[24] Since the size of DNA–AuNP conjugates is smaller than that of the Tri-AuNP DNA probe in the previous report,[8] more target DNA can be hybridized with DNA–AuNP conjugates. Therefore, the hybridization efficiency of target DNA is thus improved. Last but not least, the high stability of AuNPs is another reason for their use in the detection of DNA.

Table 1 Sequence of oligonucleotides

Oligonucleotide	Sequence (5′ → 3′)
Capture probe (probe 1)	HS-TTTTTTTTTTATGATT ATGGCTCAG
Target DNA	GGTGGATAGCAGTACCTG AGCCATAATCAT
Methylene blue probe (MB-probe 2)	GTACTGCTATCCACC-MB
MB-probe 2-SH	MB-GTACTGCTATCCACC TTTTTTTTTT-SH
MB-T10-SH	MB-TTTTTTTTTT-SH
1-mismatched DNA	GGTGGATAGCAGTACCT GAGCAATAATCAT
Completely mismatched DNA	CAACCTCAAAC AGACACCACGG



Scheme 1 Schematic illustration of the process for detection of target DNA. (A) Non-amplified detection. DNA hybridization attaches the MB probe (MB-probe 2) to the electrode surface. (B) AuNP-amplified detection. In the presence of target DNA, MB-probe 2-SH loaded on the surface of AuNPs through the Au–S bonding to form MB-probe 2-AuNP conjugates anchored at the electrode surface. (C) DNA–AuNP conjugate-amplified detection. In the presence of target DNA, MB-probe 2-SH and MB-T10-SH loaded on the surface of AuNPs through the Au–S bonding to form DNA–AuNP conjugates anchored on the electrode surface.

Experimental section

Materials

Phosphate buffered saline (PBS), 6-mercapto-1-hexanol (MCH, 97%), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, C4706) and gold(III) chloride trihydrate (99.9+%) were purchased from Sigma-Aldrich. HCl (37%) and NaCl (99.5%) were purchased from Merck. All chemicals were used as received. Ultrapure Milli-Q water (Milli-Q System, Millipore, Billerica, MA, USA) was used in all experiments. The washing buffer was 0.01 M PBS buffer (pH 7.4).

Immobilization buffer (I-buffer): 10 mM tris-HCl + 1 mM EDTA + 0.1 M NaCl (pH 7.4).

Hybridization buffer: 10 mM Tris-HCl + 100 mM KCl + 1 mM $MgCl_2$ (pH 8.0).

All oligonucleotides used here were purchased from 1st BASE Pte Ltd (Singapore) and their sequences are listed in Table 1. Prior to use, all thiolated DNA was treated with TCEP to reduce the thiol groups and thus activate the DNA. Typically, 1 μL of 20 mM TCEP solution was mixed with 5 μL of thiolated DNA (10 μM in I-buffer), and then incubated at room temperature for 30 mins.[2]

Immobilization of capture probe (probe 1) onto the Au electrode

An Au electrode (3.0 mm diameter, CHI instrument, Austin, TX, USA) was polished with alumina slurry and ultrasonicated in ethanol and then rinsed with water to get a mirror-like finish. After drying with N_2, the Au electrode was conjugated with probe 1 (see Scheme 1). Thereafter, 0.5 μL of MCH aqueous solution (100 μM) was added to the aforementioned solution, and the solution was dispensed onto the surface of the inverted Au electrode. The electrode was capped with a plastic cap to prevent the solution from drying up and incubated for 1 h at room temperature. The DNA-modified Au electrode was rinsed with washing buffer and dried with N_2.

Preparation of DNA–AuNP conjugates

AuNPs with a diameter of 13 nm were first synthesized by adding 3.5 mL trisodium citrate solution (1%) to a boiling, rapidly stirred solution of $HAuCl_4$.[21] The solution was kept boiling and stirred for 20 min. After cooling to room temperature, the prepared AuNP solution was stored at 4 °C.

AuNP was modified with DNA by adding the freshly cleaved MB-probe 2-SH and MB-T10-SH to an aqueous solution of 10 nM AuNP, where the concentrations of MB-probe 2-SH and MB-T10-SH are 3 μM and 2 μM, respectively. After 30 min, the concentrations of PBS and Tween 20 were increased to 0.01 M and 0.01%, respectively. The concentration of NaCl was increased to 0.4 M using 2 M NaCl with increments of 0.05 M NaCl every 20 min followed by 8 s of sonication. The mixed solution was incubated overnight at room temperature. Excess reagents were then removed by centrifugation at 11 000 rpm for 20 min and the precipitate was re-suspended in 100 mM PBS solution (pH 7.0). This washing step was repeated 3 times. The DNA–AuNP conjugate solution after incubation in the salting buffer displayed the same red color as the AuNP solution, suggesting that the AuNPs did not aggregate after modification of DNA (Fig. S1†).

Detection of target DNA

5 μL of target DNA with different concentrations were deposited on the surface of pre-treated Au electrodes, which were then incubated at room temperature for 1 h. After the DNA hybridization, the electrodes were rinsed with washing buffer and dried with a flow of N_2. Thereafter, 5 μL of 10 μM MB-probe 2, MB-probe 2-AuNP conjugates or DNA–AuNP conjugates were added onto the functionalized electrodes, respectively, and then incubated at room temperature for 1 h. Finally, the modified electrodes were washed and dried as before.

Measurement

The electrochemical measurements were carried out in a conventional three-electrode cell using a CHI 660C Electrochemical Workstation (CHI instrument, Austin, TX, USA). An Au electrode, Pt electrode and Ag/AgCl electrode (sat. KCl) were used as the working, counter and reference electrodes, respectively. All experiments were performed in 10 mM PBS (pH 7.4) containing 0.4 M $NaNO_3$.

Results and discussion

As a control, no peak was observed after hybridization of target DNA onto the surface of the Au electrode, *i.e.* Au-probe 1-target (black curve in Fig. 1) since no signaling molecule was assembled on the electrode. Thereafter, three different types of reporter methods were tested. As shown in Scheme 1, the MB-probe 2-SH was directly hybridized with the target DNA, giving a typical MB peak (green curve in Fig. 1). Since the number of signaling molecules was limited, the signal requires some form of amplification. Therefore, MB-probe 2-SH was conjugated onto the surface of the AuNP through the Au–S bonding and then hybridized with target DNA, resulting in a higher signal (blue curve in Fig. 1). But this amplification technique contained one-to-many recognition sites between the signal amplification unit (MB-probe 2-AuNP conjugates) and the target DNA, which decreased the sensitivity of DNA detection.[8] Therefore, another reporter, *i.e.* T10 with an MB signaling molecule (MB-T10-SH), was chosen to be conjugated onto the surface of AuNPs together with the MB-probe 2-SH, which is complementary to the target DNA while MB-T10-SH is not. Compared to the previous two methods, a significant enhancement of the MB peak was observed (red curve in Fig. 1). The higher current, obtained for the DNA–AuNP conjugates compared to the MB-probe 2-AuNP conjugates, could be due to the introduction of MB-T10-SH. The MB-probe 2-SH is longer

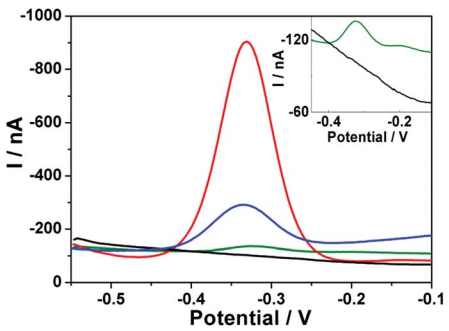


Fig. 1 DPV curves of Au-probe 1-target (black), Au-probe 1-target-probe 2 (green), Au-probe 1-target-probe 2-AuNP conjugates (blue) and Au-probe 1-target-DNA–AuNP conjugates (red). Inset: magnification of Au-probe 1-target and Au-probe 1-target-probe 2 curves. Concentration of target DNA is 1 μM. Electrolyte: 10 mM PBS (pH 7.4) containing 0.4 M $NaNO_3$; scan rate: 50 mV s^{-1}.

and more flexible than the MB-T10-SH, which could enable them to be expressed in two different putative models, *i.e.* non-interpenetrating flexible coils and rodlike configurations, respectively, on the surface of AuNPs. The rodlike configuration for MB-T10-SH would greatly increase the density of DNA on the AuNPs.[22] Based on the aforementioned description, the ratio of DNA–AuNP conjugates and target DNA has been increased from one-to-many to one-to-few, which enabled us to increase the amount of DNA–AuNP conjugates for the same amount of target DNA compared with the case of MB-probe 2-AuNP conjugates. Accordingly, the current for Au-probe 1-target DNA–AuNP conjugates increased. Therefore, the MB-probe 2-SH served two different functions in the detection process. One is to hybridize with the target DNA and the other is to be used as a signaling reporter due to the presence of MB. In addition, the MB-T10-SH is used both as a signaling reporter and spacer to decrease the interaction between target DNA and DNA–AuNP conjugates. As a result, the sensitivity of the sensor is improved. The DPV curves provided an intuitive impression of the amplification effect of DNA–AuNP conjugates, implying that they could be used to realize detection with higher sensitivity.

In order to get a good electrochemical signal, a proper ratio between the MB-probe 2-SH and MB-T10-SH should be chosen to form the DNA–AuNP conjugates. Based on our experiments, the initial concentrations of MB-probe 2-SH and MB-T10-SH were 3 and 2 μM, respectively, which produced the best signal for DNA–AuNP conjugates (Fig. S2†). The final ratio of MB-probe 2-SH and MB-T10-SH on the surface of AuNPs was calculated based on a previous report.[23] It is found that around 20–30% of the total amount of DNA on the surface of DNA–AuNP conjugates is MB-probe 2-SH, implying ~20–30 MB-probe 2-SH strands and ~70–80 MB-T10-SH on each AuNP.[23] Compared to the initial ratio between MB-probe 2-SH and MB-T10-SH, the ratio between them on the surface of AuNPs decreased greatly, which might result from the stronger affinity of MB-T10-SH to AuNPs than that of MB-probe 2-SH due to the short length of strain of MB-T10-SH. Consequently, the density of the signaling reporter DNA, *i.e.* MB-probe 2-SH, is much lower compared to the AuNPs labeled with all complementary signaling reporter DNA. This low density of MB-probe 2-SH greatly reduced the opportunity for one DNA–Au conjugate to react with many targets. It means that one DNA–AuNP conjugate only reacts with a few or even one target DNA during the detection process.

To examine the sensitivity of our assay to target DNA detection in the presence of DNA–AuNP conjugates, different concentrations of target DNA were applied (Fig. S3†) and the results are shown in Fig. 2. It was observed that the current increased when the concentration of target DNA increased from 10^{-15} to 10^{-6} M. In addition, it was found that the current is linearly related to the logarithmic concentration of target DNA across the range of 10^{-15} to 10^{-8} M (inset of Fig. 2), spanning a response region of 5 orders of magnitude. The limit of detection is estimated to be 50 fM (a signal-to-noise ratio of 3 times. Here, the noise is around 8 nA). This ultrahigh sensitivity reflected the good signal amplification of DNA–AuNP conjugates which improved the signal gain as a signal-on sensor. But in the presence of sole MB-probe 2-SH, the detection limit is 1 nM (a signal-to-noise ratio of 3 times) (Fig. S4†). Therefore, the sensitivity of target DNA detection in the presence of DNA–AuNP conjugates is 5 orders of magnitude higher than that without the AuNP amplification. This result clearly proved the large amplification effect of DNA–AuNP conjugates. When the MB-probe 2-AuNP conjugates were used, the detection limit of the sensor was around 1 pM (Fig. S5†). This result confirmed that the introduction of MB-T10-SH increased the sensitivity and lowered the detection limit of the sensor greatly. Since 5 μL of target DNA (concentration of target DNA used here is 0.5 pM) was used, an absolute detection limit of 0.25 mol target DNA (*ca.* 1.5×10^5 molecules) was achieved.

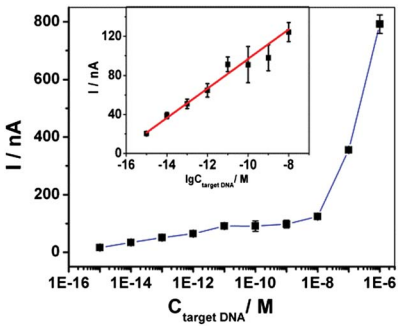


Fig. 2 Plot of current *versus* concentration of target DNA in the presence of DNA–AuNP conjugates. Inset: Linear relationship between current and logarithmic concentration of target DNA from 10^{-15} to 10^{-8} M. The error bars show the standard deviation of current measured from three parallel experiments. Electrolyte: 10 mM PBS (pH 7.4) containing 0.4 M $NaNO_3$; scan rate: 50 mV s^{-1}.

Moreover, the selectivity of the sensor was investigated in the presence of DNA–AuNP conjugates using the same concentration of target DNA, 1-mismatched DNA and the completely mismatched DNA, respectively. As shown in Fig. 3, higher current was obtained for the complementary sequence, while the current for 1-mismatched target DNA was only about 20.6% of that for target DNA of the same concentration. For the completely mismatched DNA, the signal decreased greatly as compared to the target DNA. It should be pointed out that the

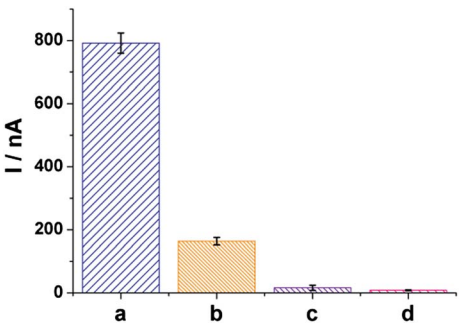


Fig. 3 Current response for different target DNA sequences in the presence of DNA–AuNP conjugates. (a) Target DNA, (b) 1-mismatched target DNA, (c) completely mismatched target DNA, and (d) background (in pure buffer). The concentration of target DNA was 1 μM. The error bars show the standard deviations of measurements taken from at least 3 independent experiments with at least 3 distinct sensors.

Table 2 Comparison of electronic DNA detection

Methods	Transducer	Detection limit	Sensor mode
DNA–Au conjugate amplification (this work)	Electrochemical	50 fM	Turn-on
Ferrocene caps on AuNP/streptavidin conjugates[28]	Electrochemical	2.0 pM	Turn-on
Polymerization-assisted electrochemical detection[29]	Electrochemical	15 pM	Turn-on
Silver-enhanced Au nanoparticle label[30]	Electrochemical	0.5 pM	Turn-on
AuNP-based detection[31]	Electrochemical	5 pM	Turn-on
Cross-linked AuNP amplification[32]	Electrochemical	100 fM	Turn-on
Au and CuS NP amplification[27]	Flow injection chemiluminescence	4.8 fM	Turn-on
Quartz crystal microbalance (AuNP)[26]	QCM	1.0 fM	Turn-off
AuNP with Ag amplification[4]	SERS	20 fM	Turn-on

good background and selectivity were obtained thanks to the sandwich strategy. The MB-probe 2 cannot hybridize with probe 1 at room temperature due to the predesigned lower melting temperature (the melting temperature of the duplex corresponding to the MB-probe 2 and probe 1 was calculated as ~ -10 °C by using the Zuker program[25]). After modification with target DNA, MB-probe 2 hybridized with it to form a stable duplex complex (the melting temperature of the complex is ~60 °C according to the Zuker program[25]). As a result, the background was low and the detection limit increased greatly. In the presence of MB-probe 2, the signal as well as the signal-to-noise ratio decreased greatly (Fig. S6†). This comparison indicates that the DNA–AuNP conjugate increased the selectivity of this electrochemical DNA sensor. In addition, the signal can be maintained well after two months (Fig. S7†), which might be due to the covalent bonding between MB and T10 and probe 2.

Table 2 lists some other methods using AuNPs to detect DNA. The sensitivity of our method has been increased greatly over the other electrochemical protocols (shown in Table 2) that involve metallic nanoparticles as labels. Only SERS,[4] quartz crystal microbalance[26] and flow injection chemiluminescence[27] were reported in literature to give better sensitivity. However, these three detection methods are more complicated than our method. For example, SERS and flow injection chemiluminescence need the secondary chemical reaction of metal deposition[4] or assembling of metal nanoparticles.[27] Metallic nanoparticle catalytic labels are required in the QCM detection.[26]

It should be pointed out that further improvement of the sensitivity to meet high-end requirements is still required, although a femtomolar sensitivity is quite impressive, which could be achieved by a more sophisticated passivation of the surface to reduce the background signal and increase the signal-to-noise ratio,[10] by employing the stem-loop structure of DNA to get greater single-nucleotide polymorphism discrimination[10,33] or by using a nanoporous electrode to get a high active surface area.[23]

Conclusions

In conclusion, the electrochemical DNA biosensor based on the DNA–AuNP conjugates shows good sensitivity and selectivity. The linear range between the current and target DNA spanned a response region of at least 6 orders of magnitude. In addition, the detection limit of target DNA as low as 50 fM was obtained. The main advantage of our method is the modification of AuNPs using two different DNA molecules as reporters. The presence of two signaling molecules decreased or even avoided cross-reaction between the target DNA and DNA–AuNP conjugates compared to the single bio-barcode of the previous reporter. We believe that more sensitive and effective DNA genosensors can be fabricated using the proper DNA reporter design.

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Notes and references

1 R. C. McGlennen, *Clin. Chem.*, 2001, **47**, 393–402.
2 J. Zhang, S. P. Song, L. H. Wang, D. Pan and C. H. Fan, *Nat. Protoc.*, 2007, **2**, 2888–2895.
3 A. A. Lubin, B. R. Baker, A. J. Heeger and K. W. Plaxco, *Anal. Chem.*, 2006, **78**, 5671–5677.
4 J. M. Nam, C. S. Thaxton and C. A. Mirkin, *Science*, 2002, **297**, 1536–1540.
5 F. Cecchini, M. Manzano, Y. Mandabi, E. Perelman and R. S. Marks, *J. Biotechnol.*, 2012, **157**, 25–30.
6 S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303–308.
7 C. H. Fan, K. W. Plaxco and A. J. Heeger, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 9134–9137.
8 H. Zhong, X. Lei, X. Hun and S. S. Zhang, *Chem. Commun.*, 2009, 6958–6960.
9 M. A. Lapierre, M. ÓKeefe, B. J. Taft and S. O. Kelley, *Anal. Chem.*, 2003, **75**, 6327–6333.
10 G. Liu, Y. Wan, V. Gau, J. Zhang, L. H. Wang, S. P. Song and C. H. Fan, *J. Am. Chem. Soc.*, 2008, **130**, 6820–6825.

11 R. E. Ionescu, S. Herrmann, S. Cosnier and R. S. Marks, *Electrochem. Commun.*, 2006, **8**, 1741–1748.
12 S. Cosnier, R. E. Ionescu, S. Herrmann, L. Bouffier, M. Demeunynck and R. S. Marks, *Anal. Chem.*, 2006, **78**, 7054–7057.
13 J. Zhang, S. P. Song, L. Y. Zhang, L. H. Wang, H. P. Wu, D. Pan and C. H. Fan, *J. Am. Chem. Soc.*, 2006, **128**, 8575–8580.
14 F. A. Wang, J. Elbaz, C. Teller and I. Willner, *Angew. Chem., Int. Ed.*, 2011, **50**, 295–299.
15 R. Z. Fu, T. Li, S. S. Lee and H. G. Park, *Anal. Chem.*, 2011, **83**, 494–500.
16 J.-J. Zhang, T. T. Zheng, F.-F. Cheng and J.-J. Zhu, *Chem. Commun.*, 2011, **47**, 1178–1180.
17 A. E. Prigodich, A. H. Alhasan and C. A. Mirkin, *J. Am. Chem. Soc.*, 2011, **133**, 2120–2123.
18 D. Du, Z. X. Zou, Y. Shin, J. Wang, H. Wu, M. H. Engelhard, J. Liu, I. A. Aksay and Y. H. Lin, *Anal. Chem.*, 2010, **82**, 2989–2995.
19 N. L. Rosi and C. A. Mirkin, *Chem. Rev.*, 2005, **105**, 1547–1562.
20 J.-M. Nam and C. A. Mirkin, *Science*, 2003, **301**, 1884–1886.
21 J. W. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246–252.
22 A. B. Steel, R. L. Levicky, T. M. Herne and M. J. Tarlov, *Biophys. J.*, 2000, **79**, 975–981.
23 K. C. Hu, D. X. Lan, X. M. Li and S. S. Zhang, *Anal. Chem.*, 2008, **80**, 9124–9130.
24 C. S. Thaxton, H. D. Hill, G. Georganopoulou, S. I. Stoeva and C. A. Mirkin, *Anal. Chem.*, 2005, **77**, 8174–8178.
25 N. R. Markham and M. Zuker, *Nucleic Acids Res.*, 2005, **33**, W577–W581.
26 Y. Weizmann, F. Patolsky and I. Willner, *Analyst*, 2001, **126**, 1502–1504.
27 S. S. Zhang, H. Zhong and C. F. Ding, *Anal. Chem.*, 2008, **80**, 7206–7212.
28 J. Wang, J. H. Li, A. J. Baca, J. B. Hu, F. M. Zhou, W. Yan and D.-W. Pang, *Anal. Chem.*, 2003, **75**, 3941–3945.
29 Y. F. Wu, S. Q. Liu and L. He, *Anal. Chem.*, 2009, **81**, 7015–7021.
30 H. Cai, Y. Q. Wang, P. G. He and Y. Z. Fang, *Anal. Chim. Acta*, 2002, **469**, 165–172.
31 L. Authier, C. Grossiord, P. Brossier and B. Limoges, *Anal. Chem.*, 2001, **73**, 4450–4456.
32 D. Li, Y. M. Yan, A. Wieckowska and I. Willner, *Chem. Commun.*, 2007, 3544–3546.
33 G. Bonnet, S. Tyagi, A. Libchaber and F. R. Kramer, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 6171–6176.